potential upstroke. These data are the first report of FGF13 as regulator of Nav1.5 in heart and suggest that FGF13 isoform-specific regulation of cardiac Nav channels plays important physiological and pathophysiological roles.

2279-Pos Board B265

CaMKII Regulates Cardiac Sodium Channel Nav1.5 by Phosphorylation in the Loop Between Domain I and II

Anthony W. Herren, Nicole M. Ashpole, Kenneth S. Ginsburg, Donald M. Bers, Andy Hudmon.

The cardiac Na channel $Na_V1.5$ current (I_{Na}) is critical to cardiac excitability and altered I_{Na} gating has been implicated in genetic and acquired arrhythmias. Ca-calmodulin dependent protein kinase (CaMKII) is upregulated in heart failure and has been shown to cause I_{Na} gating changes that mimic a point mutant in humans associated with combined long QT and Brugada syndromes. We sought to identify the CaMKII phosphorylation target site on $Na_V1.5$ that mediates the CaMKII-induced alterations in I_{Na} gating. We analyzed CaMKII-dependent phosphorylation of $Na_V1.5$ intracellularly accessible sites using a series of GST-fusion constructs, immobilized peptide arrays, and soluble peptides. CaMKII phosphorylation sites were identified at S516 and S593/T594 in the intracellular $Na_V1.5$ loop between domains 1 and 2. Wild-type (WT) and phospho-mutant human Nav1.5 were co-expressed with CaMKIIδ_C-GFP in HEK293 cells and I_{Na} was recorded by whole-cell patch clamp under pipette conditions that acutely activated CaMKII (+1 μM free [Ca], + 1 μM Calmodulin). As observed in myocytes, CaMKII shifted WT I_{Na} availability to more negative membrane potentials and enhanced accumulation of I_{Na} into intermediate inactivation, but these effects were abolished by mutating either of the CaMKII sites to non-phosphorylatable alanine residues ($V_{1/2}$ of availability: WT −101.8 ± 4.5 (n=10); S593/T594AA −89.7 ± 3.5 (n=11) ; S516A −93.0 ± 5.5 (n=18)). Furthermore, the phospho-mutant channels mimicked WT I_{Na} treated with 1 μM AIP, a specific CaMKII inhibitor, and AIP had no effect on phospho-mutant I_{Na}. CaMKII activation had no effect on the $V_{1/2}$ of activation or I-V curve shape of any channel. CaMKII-dependent phosphorylation of $Na_V1.5$ at multiple sites (including Ser593/Thr594 and Ser516) appears to be required to evoke loss-of-function changes in gating that could contribute to acquired Brugada syndrome like effects in heart failure.

2280-Pos Board B266

Chlorobutanol, a Pharmaceutical Preservative and Sedative Hypnotic, Inhibits Brain Type Voltage Gated Sodium Channels

George Kracke, Alice Landrum.

Introduction- Chlorobutanol (1,1,1-trichloro-2-methyl-2-propanol) is a widely used chemical preservative for injectable drugs, eye drops, mouth washes, and cosmetics. It is an analgesic, experimental general anesthetic, and sedative hypnotic in man. The mechanism of action of chlorobutanol is unclear. Our goal was to test whether chlorobutanol inhibits brain type voltage gated sodium channels.

Methods- Mammalian Na_v 1.2 voltage gated sodium channels were expressed in *Xenopus* oocytes by injecting the alpha subunit cRNA. Inward sodium currents were measured with two-electrode voltage clamp using standard depolarization protocols. The study was approved by our institutional animal care and use committee.

Results- Chlorobutanol was tested at concentrations ranging from 0.03 to 10 mM. Chlorobutanol reversibly inhibited closed, resting channels and inactivated channels in a concentration dependent manner. The voltage dependence of activation was shifted in the depolarizing direction. Fast inactivation voltage dependence was not affected. There was little state dependent block and no apparent use dependence.

Discussion and Conclusions- Chlorobutanol inhibited Na_v 1.2 channels at concentrations less than those used to preserve pharmaceuticals. Its use as a preservative in injectable methadone and local anesthetic solutions, and in eye drops likely adds an analgesic component to these solutions.

2281-Pos Board B267

Enhancement of Slow Inactivation of Voltage-Gated Na^+ Channels by Ranolazine

Ryoko Hirakawa, Lynda V. Liu, John C. Shryock, Luiz Belardinelli, Sridharan Rajamani.

Ranolazine is an anti-anginal drug that blocks cardiac ($Na_v1.5$) late Na^+ current (I_{Na}) at therapeutic concentrations (2-10 μM). Recent electrophysiological studies have shown that ranolazine also blocks skeletal muscle ($Na_v1.4$) and neuronal ($Na_v1.1$, $Na_v1.7$, $Na_v1.8$) I_{Na}. We investigated the effects of ranolazine on peak I_{Na} and on both sustained repetitive firing (SRF) and 0 mM Mg^{2+}- induced (0 $[Mg^{2+}]_O$) continuous high frequency firing of action potentials in cultured rat hippocampal neurons. Ranolazine caused a voltage (−60 mV) and frequency (10 Hz)-dependent inhibition of I_{Na} with IC_{50} values of 0.48 and 61.8 μM (n=4-6), respectively. Ranolazine (10 μM, n=4) did not shift the voltage-dependence of steady-state fast inactivation; however, it caused a 15-mV hyperpolarizing shift ($p<0.05$, compared to control, n=4) in the slow inactivation. Consistently, ranolazine (10 μM) reduced SRF only during a 4-sec burst but not with a 1-sec depolarization step (Fig.1). Furthermore, ranolazine (10 μM) reduced 0 $[Mg^{2+}]_O$ -induced high frequency firing of spontaneous action potentials from 0.78 to 0.45 Hz ($p<0.05$, n=5). Taken together, these data suggest that ranolazine suppresses the propagation and conduction of action potentials by preferentially interacting with Na^+ channels in the slow inactivated state.

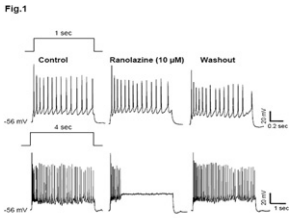

Fig. 1: Representative tracings of 1 and 4 sec sustained repetitive firing of action potentials in cultured hippocampal neurons in the absence (control) and presence of ranolazine (10 μM) followed by washout.

2282-Pos Board B268

Y1767C, a Novel *SCN5A* Mutation Induces a Persistent Sodium Current and Potentiates Ranolazine Inhibition of $Na_V1.5$ Channels

Hai Huang, Silvia G. Priori, Carlo Napolitano, Michael E. O'Leary, **Mohamed Chahine**.

Long QT syndrome type 3 (LQT3) has been traced to mutations of the cardiac Na channel ($Na_v1.5$) that produce persistent Na currents leading to delayed ventricular repolarization and Torsades de pointes. We performed mutational analyses of patients suffering from LQTS and characterized the biophysical properties of the mutations that we uncovered. One LQT3 patient carried a mutation in the *SCN5A* gene in which the cysteine was substituted for a highly conserved tyrosine (Y1767C) located near cytoplasmic entrance of the $Na_v1.5$ channel pore. The wild-type and mutant channels were transiently expressed in tsA201 cells, and the Na currents were recorded using the patch-clamp technique. The Y1767C channel produced a persistent Na current, more rapid inactivation, faster recovery from inactivation, and an increased window current. The persistent Na current of the Y1767C channel was blocked by ranolazine but not by many class I antiarrhythmic drugs. The incomplete inactivation, along with the persistent activation of Na channels caused by an overlap of voltage-dependent activation and inactivation, known as window currents, appeared to contribute to the LQTS phenotype in this patient. The blocking effect of ranolazine on the persistent Na current suggested that ranolazine may be an effective therapeutic for treating patients with this mutation. Our data also revealed the unique role for the Y1767 residue in inactivating and forming the intracellular pore of the $Na_v1.5$ Na channel.

2283-Pos Board B269

The Selective $Na_v1.8$ Sodium Channel Blocker A-803467 Affects Electrical Activity in Intracardiac Neurons, but not in Cardiomyocytes

Marieke W. Veldkamp, Carol A. Remme, Brendon Scicluna, Cees A. Schumacher, Rianne Wolswinkel, Antoni C.G. van Ginneken, Connie R. Bezzina, Arie O. Verkerk.

Background. Recently we observed differential myocardial expression of the brain-type sodium channel isoform *Scn10a* between two inbred mouse strains, both harboring the Scn5a-1798insD+/- mutation and displaying different severity of conduction disease. The functional role of *Scn10a* in the heart is as yet unknown, and we therefore investigated expression and channel activity of $Na_V1.8$ (encoded by *Scn10a*) in intracardiac neurons and myocardium of the murine heart.

Methods. Immunocytochemistry was performed using anti-$Na_V1.8$ antibody on mouse embryos and adult murine cardiac tissue sections. The effect of the $Na_V1.8$ blocker A-803467 (500 nM) on action potentials (AP's) and sodium current (I_{Na}) properties was assessed in isolated intracardiac neurons and ventricular myocytes.

Results. In embryonic and adult heart tissue sections, $Na_V1.8$ staining was observed at the epicardial surface, and within the myocardium in between cardiomyocytes. The $Na_V1.8$ blocker A-803467 had no effect on either mean I_{Na} density or I_{Na} kinetic properties in isolated myocytes, but clearly reduced I_{Na} density in intracardiac neurons (−344 ± 51 pA/pF versus control −448 ± 61; mean ± SEM, n=11). In addition, the slow component of the current decay (τ_{slow}) at −20 mV was accelerated in the presence of A-803467 (2.8 ± 0.3 ms versus control 3.4 ± 0.4 ms; mean ± SEM, n=5) and $V_{1/2}$ of voltage-dependent inactivation was shifted by −9.6 mV (−73.6 ± 2.0 mV versus control −64.0 ± 1.6 mV; mean ± SEM, n=5). This is consistent with a reduction in slowly inactivating brain-type sodium current with depolarized voltage-dependent inactivation. In AP measurements A-803467 did not affect cardiomyocyte upstroke velocity, but reduced AP firing frequency in intracardiac neurons by 50%.

Conclusion. The sodium channel $Na_V1.8$ is expressed in murine heart, and is functionally present in intracardiac neurons, but absent in cardiomyocytes. Thus, $Na_V1.8$ may influence myocardial electrophysiological properties through its contribution to cardiac neuronal activity.

2284-Pos Board B270

Turret Histidines in pH Modulation of the Cardiac Voltage-Gated Sodium Channel

David K. Jones, Thomas W. Claydon, Peter C. Ruben.

Low pH reduces single channel conductance and destabilizes the inactivated states of the cardiac voltage gated sodium channel (NaV1.5) by increasing window current and inhibiting use dependent inactivation. Outer ring carboxylates are believed to underlie proton block. In contrast, the molecular underpinnings by which protons modulate NaV1.5 channel kinetics (Jones et al. 2010) are unknown. We hypothesize that turret histidines found in domain II, and conserved in all NaV channel isoforms, play a pivotal role in pH modulation and channel function, as has been demonstrated in potassium channels (Claydon et al. 2002). We expressed wild type and mutant NaV1.5 channels in Xenopus oocytes and recorded currents using a cut-open voltage clamp with extracellular solution titrated to either pH 7.4 or pH 6.0. Replacement of a histidine with a glutamine at position 880 abolished the effect of pH on window current and use dependent inactivation. Additionally, the H880Q mutant depolarized the voltage dependence of activation and fast inactivation. We therefore propose H880 plays an important role in pH modulation and function of NaV1.5.

2285-Pos Board B271

Palmitoylation Affects the Interaction of Animal Toxins with Sodium Channels

Frank Bosmans, Mirela Milescu, Kenton J. Swartz.

Animal toxins can interact with voltage sensor paddle motifs within voltage-activated sodium (Nav) channel voltage sensors to alter channel function. These structural motifs were originally identified in voltage-activated potassium (Kv) channels, where they were shown to move at the protein-lipid interface to drive activation of the voltage sensors and opening of the pore. Serendipitously, we identified a variant of Nav1.2 with functional properties similar to wild-type, but that exhibits a 20-fold higher apparent affinity for PaurTx3, a voltage sensor toxin isolated from tarantula venom. The amino acid difference that underlies this large divergence in toxin sensitivity was identified as G1079C and is located in the intracellular loop between domain II and domain III of the channel. Since this region is unlikely to be directly accessible to peptide toxins applied to the external solution, we explored the possibility that cysteine palmitoylation underlies this observation. When we inhibit palmitoylation of Nav1.2 and the G1079C mutant by using 2-Br-palmitate, the apparent affinities of the toxin for both channels now coincide. While surveying other molecules, we found a second tarantula toxin, ProTx-II, which is similarly influenced by Nav1.2 palmitoylation. Interestingly, both these toxins interact with the paddle motif in domain II of the channel whereas a scorpion toxin that interacts with the domain IV voltage sensor, AaHII, is not affected. These results suggest an important role for palmitoylation in shaping the pharmacological properties of Nav1.2 and have implications for changes in drug susceptibility of Nav channels caused by intracellular mutations.

2286-Pos Board B272

Molecular Determinants for Alpha-Scorpion Toxin Binding to the Resting State of a Voltage Sensor of Brain Sodium Channels

Jinti Wang, Vladimir Yarov-Yarovoy, Roy Kahn, Dalia Gordon, Michael Gurevitz, Todd Scheuer, William A. Catterall.

Voltage-gated sodium channels are responsible for initiation and propagation of action potentials in nerve and muscle. α-Scorpion toxins, including LqhII (*Leiurus quinquestriatus hebraeus, type II*), bind to the extracellular face of the sodium channel and inhibit fast inactivation. Previous work has identified E1613 (rat $Na_V1.2$) in the S3-S4 loop of domain IV as a key site of toxin binding, but antibody-mapping studies suggested additional components of the toxin receptor site in domains I and IV. To identify other determinants of toxin action, extracellular amino acid residues in domains I and IV of the α subunit were converted to alanine or other neutral amino acids, and mutant channels were transiently expressed in tsA-201 cells and tested for channel function and toxin action by whole-cell voltage clamp. Toxin affinity for most mutant channels was unchanged from wild-type. However, electrophysiological analysis showed that T393A, T1560A, F1610A, and E1613A, had 3.4-, 5.9-, 10.7-, and 3.9-fold lower affinities for LqhII, respectively. Most of these mutations increased the rate of toxin dissociation, but had little effect on the rate of toxin association. These results indicate that T1560 in the S1-S2 loop, F1610 in the S3 segment, and E1613 in the S3-S4 loop in domain IV form one aspect of the toxin-binding site; T393 in the domain I SS2-S6 loop may contribute to a second part of the site. Modeling the toxin receptor site and the toxin-receptor interaction with the Rosetta-Membrane structural-modeling and ligand-docking algorithms resulted in a three-dimensional model of LqhII binding to the voltage sensor of the channel. This model refines the structure of the resting state of the voltage sensor and suggests its mode of interaction with a gating modifier toxin. Supported by U01 NS058039.

2287-Pos Board B273

High-Resolution Structural Modeling of Voltage-Dependent Conformational Changes in the Voltage Sensor of NaChBac

Vladimir M. Yarov-Yarovoy, Paul DeCaen, Todd Scheuer, David Baker, William A. Catterall.

Progress has been made in determining high-resolution structures of voltage sensors of voltage-gated ion channels in activated and/or inactivated states. However, high-resolution structures of resting and intermediate states of voltage sensors remain unknown. We constructed high-resolution structural models of resting, intermediate, and activated states of the voltage-sensing domain (VSD) of the bacterial sodium channel NaChBac using the Rosetta-Membrane computational method, the Rosetta method-based FoldIt program, the x-ray structure of the Kv1.2-Kv2.1 chimeric channel, and experimental data demonstrating sequential interactions between gating-charge-carrying arginines in S4 segment and negatively charged residues in S1, S2, and S3 segments during activation. The resulting sliding helix model suggests that the S4 is a 3-10 helix from the first or second gating-charge-carrying arginine to the fourth gating-charge-carrying arginine or S125 near its C-terminus during the conformational change between the resting and activated states. The S4 segment slides ~10 Å through a narrow groove formed by rigid S1, S2 and S3 segments, rotates ~30-60 degrees with respect to its own axis, and tilts sideways at a pivot point formed by a highly conserved hydrophobic region in the middle of the VSD. During S4 movement, gating-charge-carrying arginines sequentially form ion pairs and hydrogen bonds with highly conserved negatively charged and polar residues in the narrow gating pore and in the intracellular and extracellular water-accessible cavities of the VSD. Conformational changes of the intracellular half of S4 are coupled to lateral movement of the S4-S5 linker that leads to movement of the intracellular half of S5 and S6 segments and either opens or closes the intracellular gate of the ion-conducting pore. Supported by NIH R01 NS015751 to W.A.C. and P20GM076222 to D.B.

2288-Pos Board B274

Disulfide Locking Reveals Interaction of the Gating Charges with a Negative Charge in the S1 Segment During Activation of the NaChBac Voltage Sensor

Paul G. DeCaen, Vladimir Yarov-Yarovoy, Todd Scheuer, William A. Catterall.

Opening and closing the pore of voltage-gated ion channels are mechanically linked to conformational movement of the positively charged fourth transmembrane segment (S4) in the voltage sensor. Disulfide locking of cysteines substituted for gating charges and for E43 at the extracellular end of the S1 segment of the bacterial sodium channel NaChBac demonstrated that a threonine at gating charge position 0 (T0) and arginine at the first gating charge position (R1) located at the extracellular end of S4 interact with E43 in the closed state, whereas the second and the third gating charges (R2) and (R3) interact with E43 in activated states. The kinetics and voltage dependence of disulfide locking of R2C and R3C demonstrate sequential interactions of the gating charges with E43C during voltage sensor activation. We did not observe disulfide locking with the fourth or last gating charge (R4) and E43, suggesting that we have described the upper limit to the outward movement made by the S4 during channel activation. These results indicate that S4 moves 8-10Å outward with respect to E43 in the transition from resting to activate states, which supports a sliding helix model of voltage sensor movement in voltage-gated ion channels.

2289-Pos Board B275

An Alternative Tetramerisation Domain Restores Expression of the NAChBac Voltage-Gated Sodium Channel

Andrias O. O'Reilly, Andrew M. Powl, B.A. Wallace.

The NaChBac voltage-gated sodium channel from Bacillus halodurans is a homo-tetramer, which matches the quaternary structure of potassium channels but contrasts with the single-chain mammalian sodium channels. We have previously demonstrated that the cytoplasmic C-terminus of NaChBac functions as a channel assembly domain [Powl et al (2010) PNAS 107:14064-14069]. Sequential deletion mutations reduce the amount of NaChBac in the membrane fraction, with removal of the entire C-terminus eliminating the channel assembly. A similar effect was also found upon removal of potassium channel assembly domains. However, potassium channel expression can be rescued by using an alternative tetramerisation domain replacement.

In this study we generated a chimeric form of NaChBac in which the C-terminus was removed and the short cytoplasmic N-terminus was replaced with the